

Public Abstract

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Title:Bacterial ribosome conformational dynamics during translation termination and rescue

Structural and biochemical studies of class I release factors (RF1 and RF2) and non-stop complex rescue protein YaeJ bound to the ribosome have made important contributions to understanding the mechanism of peptidyl-tRNA release and ribosome recycling. However, the dynamic conformational changes that result from the interaction of these factors with the ribosome have yet to be elucidated in significant detail. Here, we employed single molecule Förster resonance energy transfer (smFRET), to investigate the influence that wild-type and mutant versions of these proteins exert on the conformation of the ribosome. We prepared fluorescently labeled ribosomes in the post-hydrolysis state with various mRNA sequences designed specifically for efficient binding of either of these factors. We found that the class I RFs stabilized the ribosome in the non-rotated state only upon effective binding to a stop codon in the A-site. In contrast, YaeJ was only able to stabilize the non-rotated state when truncated mRNA was present within the ribosome. These observations clearly demonstrate the different requirements for translational termination versus ribosome rescue. We propose that interactions of these proteins with bridge B2 facilitate stabilization of the ribosome in the non-rotated state and that this has the most profound influence on subunit rotation that we investigated. Finally, our results are consistent with the idea that stabilization of the ribosome in the non-rotated state is necessary for subsequent factors to bind and preparing the ribosome for recycling. We believe that the community of molecular biologists, biochemists, and biophysicists who are studying translation termination and ribosome rescue, as well as investigators who are interested in protein synthesis and ribonucleoprotein structure and function will find our findings of great interest.